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CHEMICAL & ENGINEERING

NEWS

New reports on danger of carbon dioxide buildup
Page 22

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Toxicity Testing

Developing alternatives to animal use

Page 7

third quarter, barring companies that went from deficits to profits, was at Reichhold Chemicals. Reichhold's earnings from continuing operations increased 400% from third-quarter 1982 to $5.5 million. However, the earnings gain weighing most in the industry totals was from Dow Chemical, whose profits rose 146% to $86.0 million. Without Dow, the overall increase would have been just 27.5%, almost 10 percentage points lower.

Dow's president and chief executive officer, Paul F. Oreffice, says, "We have come a long way from the dismal economic conditions that the chemical industry faced only a year ago. Even though the nine-month earnings are lagging 1982, our core businesses are in far better shape versus third-quarter 1982 and we expect continued improvement." Earnings from operations at Dow, excluding extraordinary items, are 12% ahead of 1982 through the first three quarters.

Another company happy with the chemical economy is Celanese, whose chairman, president, and chief executive officer, John D. Macomber, says, "The U.S. economic expansion is continuing, and domestic demand has picked up for our chemicals, fibers, and specialty products in the automotive, housing, and other industries. We achieved a profit this quarter for the first time in a year."

A "we are pleased" comment also comes from Du Pont chairman Edward G. Jefferson, who at C&EN press time disclosed a 60% earnings jump for the company in the third quarter from 1982. Du Pont's chemical earnings more than doubled on an 11% physical volume increase. Its chemical plants ran at 72% of capacity, up from 67% in third-quarter 1982 and 62% in early 1983.

All told, the economic improvement bodes well for the chemical industry this year. Although total profits are still not fully recovered, another big gain in the fourth quarter should do the trick. Such a gain is in the bag, since the comparison will be with the worst recession quarter of 1982. And unless the general U.S. economy falls apart, a banner year is in store for the chemical industry in 1984. □

Senate kills Clinch River breeder reactor

The rollercoaster career of the Clinch River breeder reactor project is finally over. The Senate, which has been keeping the project alive for years, voted last week to delete the $1.5 billion earmarked for the project from the fiscal 1984 supplemental appropriations bill. The House, where opposition to the project is intense, didn't even bother to consider funding it this year.

The reactor, to be sited on the Clinch River in southeastern Tennessee, was aimed at demonstrating the liquid-metal fast breeder reactor, which would produce more plutonium fuel than it used while generating electricity. The electricity was to be supplied to the Tennessee Valley Authority.

The project has been plagued by cost overruns for years and there have been recent assertions that its technology is by now outdated. When the demonstration reactor was first authorized in 1971, the cost was put at $400 million, with more than half to be supplied by electric utility companies. The most recent cost estimate is $4.5 billion, with utilities expected to provide $1 billion, which they have seemed reluctant to do.

The Department of Energy seems to have bowed to the inevitable, saying that it is disappointed with the vote but will begin an orderly termination of the project on which $1.7 billion already has been spent for design and engineering work, excavation, and some major components. DOE estimates that closing the project down will cost $150 million to $350 million. □

Quality of science students may have improved

The quality of science and engineering students—both undergraduate and graduate—seems not to have changed much over the past five years, and there is some indication the quality may have improved. That's the overall impression from a survey of senior academic officials conducted for the National Science Foundation by the American Council on Education.

Judgments of quality were obtained from a sample of 486 institutions with four-year undergraduate programs and 383 with graduate programs. No attempt was made to distinguish between science and engineering students or between students in various science or engineering fields.

Specifically, 60% of those officials queried reported "no change" in quality of undergraduate or graduate students. Most of the rest, however, expressed views indicating improvement, with 25% saying "significant improvement" among science and engineering undergraduates and 15% saying "significant decline." For graduate students, 28% of the graduate deans surveyed saw an improvement in quality and only 12% reported declines.

The survey asked for subjective judgments, but it did ask also for the officials to provide the bases for

Graduate deans rate five-year change in applicant quality

Type of institution	No significant change	Significant improvement		Significant decline	
		Overall	In a few fields	Overall	In a few fields
All institutions	60%	10%	18%	6%	6%
Doctorate granting	59	11	16	5	9
Nondoctorate granting	61	9	20	7	3
Publicly controlled	58	13	17	6	6
Privately controlled	63	5	21	5	6
50 largest science/engineering doctorate	61	6	21	4	9

Note: Opinions of graduate deans about change in quality of applicants for graduate study in science/engineering fields over the five years to 1982. Sources: National Science Foundation, American Council on Education

their conclusions. These answers show that many different bases were used, especially among the groups that formed different judgments.

For example, at the undergraduate level, the principal indicators cited as the basis for opinions were faculty impressions, 32%; trends in achievement test scores, 29%; high school grades or class rank, 21%; requirements for remedial courses in mathematics, 12%; and other factors, 6%. Different indicators tended to be used by those reporting no change, improvement, or decline.

At the graduate level, there was a similar pattern, with graduate deans basing their opinions on faculty perceptions, 29%; undergraduate grade-point averages, 28%; trends in applications from graduates of high-quality undergraduate institutions and Graduate Record Examination scores, 20% each; and other factors, 3%. □

U.K. considers sites for nuclear waste disposal

Radioactive waste may end up in an unused anhydrite mine belonging to the U.K.'s Imperial Chemical Industries. The site, beneath ICI's major chemical complex at Billingham on England's northeast coast, is one of two being considered. The other is a thick clay deposit in Bedfordshire north of London.

The locations have been selected by the U.K.'s Nuclear Industry Radioactive Executive. NIREX was set up last year by British Nuclear Fuels Ltd., the U.K. Atomic Energy Authority, and the country's electricity generating boards to coordinate plans for the management and disposal of low- and intermediate-level radioactive wastes resulting from the U.K.'s nuclear activities.

The next step will entail thorough field studies of the sites' potential suitability for construction of radioactive waste depositories. Paramount will be an evaluation of the geology of the areas. This will take several years to complete.

If the sites prove suitable, NIREX will seek planning permission to move ahead with the work. The hope is that the Bedfordshire repository could be ready to receive waste by the end of the decade. It could accommodate some 353,000 cu ft a year of short-life, medium-level material incorporated in concrete or resin, and packaged in steel or concrete drums. These would be buried in a series of trenches and covered with the thick clay. The Billingham mine, about 750 feet below ground level, has a potential capacity of 388 million cu ft. It would be the repository of long-life, medium-level waste.

Not surprisingly, people who live and work in the vicinity of the two areas are greatly concerned at the prospect of radioactive material being dumped there. Paul Marsden, deputy chairman of ICI's agricultural division, "regrets" the selection of Billingham. "We would like to be left alone to do the things we are good at: making chemicals," he says. □

Toxicity lab officials found guilty of fraud

A U.S. district court jury in Chicago has found three former officials of Industrial Bio-Test Laboratories guilty of multiple counts of fraud, stemming from falsification of data obtained in four animal toxicity tests conducted by the firm (C&EN, June 29, 1981, page 5). Meanwhile, officials of the National Toxicology Program have expressed doubts about the validity of toxicity tests conducted by other laboratories.

In a joint trial, after seven months of complex testimony and 11 days of jury deliberation, Moreno L. Keplinger, former manager of toxicology at Bio-Test, was found guilty of six of eight counts charging alleged mail or wire fraud, or false statements made to government agencies. James L. Plank, a former assistant to Keplinger, was found guilty of five counts. Paul L. Wright, former section head for rat toxicology, was found guilty of three counts. Each of the counts carries a maximum sentence of five years in prison. No date for sentencing has been set; post-trial motions for acquittal will be argued next Feb. 17.

Joseph C. Calandra, former president of the Northbrook, Ill.-based firm, also had been indicted and brought to trial with the others. In his case, however, a mistrial was declared in July, to allow him to undergo heart surgery. Whether he will be retried has not yet been decided.

In the late 1960s and early 1970s, Bio-Test, then a subsidiary of Nalco Chemical, was a leader in the fast-growing field of toxicity testing. But a 1976 inspection by Food & Drug Administration officials revealed deficiencies in test procedures. Thereafter, things fell apart at Bio-Test. In 1977 the firm admitted to "material defects" in rodent studies. A number of previously approved drugs and pesticides had to be retested. Customers brought suits. Bio-Test sold most of its facilities, but a skeleton staff remains in Northbrook to tidy up loose ends.

The 1981 criminal indictment (and 1983 trial) dealt with tests of four compounds: naproxen, an antiarthritic drug; trichlorocarbanilide, an antibacterial; a herbicide; and a pesticide. Safety of all four products was reconfirmed after retests or audits.

In another development, officials of the National Toxicology Program say they plan to review 166 unpublished studies made by several laboratories. They also may audit some of another 250 studies, results of which already have been published.

The action was prompted, NTP says, by one allegedly flawed test of the carcinogenicity of methylene chloride, made by Gulf South Research Institute, a not-for-profit organization based in Baton Rouge, La. The findings in that case convinced program officials that they should review not only 25 other studies by Gulf South but all the other unpublished studies made for the program by other laboratories. NTP stresses that there is no suggestion of fraud in the Gulf South case, just "sloppy science."

Gulf South president James H. Clinton concedes that the lab has had some problems and has made some changes. But, he argues, the study, made from 1978 to 1980, is now being judged by stricter 1983 standards. □

Alternative Methods Could Cut Animal Use in Toxicity Tests

Scientists are encouraged by preliminary results from cell culture and other techniques to assess eye irritation and various types of toxic effects from chemicals

Ron Dagani
C&EN Washington

A dog with large, liquid eyes stares through the bars of his cage. Above this photograph the caption reads: "Would you pay someone to kill this animal?"

This advertisement and others like it that ran in several newspapers this year, attacks a test called the LD_{50}, which is used to determine the acute toxicity of chemicals in animals. The test "causes agonizing death for millions of lab animals," the ad states, "and you pay for it!"

Media campaigns like this one, designed and carried out by well-financed animal welfare groups, have galvanized the general public and the scientific and regulatory communities into a greater awareness of the widespread use—some would say misuse—of animals in research and testing of chemicals and consumer products.

With funding from animal rights groups, corporations, foundations, and others, scientists have intensified their efforts to find and validate alternative tests and research methods that do not subject animals to suffering—or better yet—do not use animals at all.

It is a tall order. Animals have played a crucial role in toxicology, biomedicine, and other fields of science for as long as bench chemists have depended on test tubes and beakers. About 70 million animals are used each year in the U.S. for toxicity testing and biomedical and behavioral research, according to the Office of Technology Assessment. The vast majority of these are mice and rats. But the rabbits, cats, dogs, monkeys, and other higher animals used number in the hundreds of thousands.

Some 20% of all these animals, about 14 million, are used in toxicity testing, an enormous enterprise aimed at protecting humans from the chemical web that is an inextricable part of modern life. Whether they are used to evaluate the potential eye irritancy of a new shampoo, or the possible carcinogenicity of an industrial chemical, laboratory animals are the unwilling stand-ins for humans.

Many believe that humans can be protected adequately without resorting to such extensive use of live animals. The alternatives include the use of tissue and cell cultures, isolated tissues and organs, simulated tissues and body fluids, and bacteria. These are known as in vitro methods. In addition, computers are playing an increasingly important role in helping scientists develop mathematical models and quantitative structure-activity relationships that can identify potential chemical troublemakers.

The current drive to reduce animal use or find replacements for animals in the lab, particularly in the testing of consumer products, is motivated by several factors. First and foremost is the animal welfare movement. Animal activists have questioned the mo-

rality of using other species to benefit humans. They also have deftly played to the hearts of the public, tying into many people's feeling of kinship to animals.

But the practical shortcomings of animal tests also have served as an impetus. Lab animals have become expensive, and the data they provide tend to be imprecise and sometimes of limited usefulness. Cell culture systems, for example, are simpler and cheaper than using animals. And in light of recent advances made in such in vitro methods, these alternatives hold out the promise of providing scientists with more precise quantitative data.

None of these potential alternatives, however, offers a panacea. "Many of the whole-animal tests now used to measure irritancy or systemic and neurotoxicity rely on the interaction of a multitude of organ functions and [chemical] products," explains Dennis M. Stark, head of the Laboratory Animal Research Center at New York's Rockefeller University. "This interaction is not going to be easily measured in isolated culture systems." Stark, along with many other researchers, believes that many alternative bioassays may be necessary, as a battery, to obtain the same information elicited from a whole-animal test.

During the past few years, the animal rights movement has pummeled the consumer products industry especially hard. It has taken aim at two specific tests—the Draize and LD_{50}. Besides stimulating research into a variety of alternatives, these efforts have succeeded in reducing the numbers of animals used in toxicity testing. Thus far, however, no proven, comprehensive replacements for these animal tests have emerged, but researchers are encouraged by their preliminary results.

The Draize test, named after the pharmacologist who standardized it in 1944, is widely used by manufacturers to determine the potential eye irritancy of a plethora of consumer, pharmaceutical, and industrial products. The test substance is placed in one eye of an albino rabbit; the other eye, untreated, serves as the control. An irritant will cause inflammation of the treated eye. The degree of inflammation is assessed visually by comparing the appearance of the eye to standardized photographs.

To the nonscientist, the idea of using "cute bunny rabbits" to test potentially eye-irritating, and hence painful and possibly blinding products, may seem downright diabolical. But the choice is dictated by scientific logic. The rabbit's eye, being thinner than a human's, also is more sensitive. Also, the animal's tear glands are not as effective as a human's in washing away irritants. And because an albino rabbit's eyes contain virtually no pigment, it's easier to see inflammation or damage when it occurs.

The procedure used for rating the degree of inflammation and damage caused by a substance in the Draize test is extremely subjective, however. Several studies published during the 1970s showed that the use of the test to pigeonhole substances as either mild, moderate, or severe irritants is subject to much variability. As one scientist tells C&EN, "Pink to you might be red to me."

These results served as ammunition for animal advocates like Henry Spira, who charged that the Draize test was used indiscriminately and that it was repeated too often with known irritants. Spira pulled together a coalition of more than 400 groups that, despite their individual agendas, were adamant in their opposition to the Draize test. Revlon, a major user of the test and a giant in the glamorous world of cosmetics, was attacked in full-page newspaper ads and beseiged by crowds of protesters marching past the front door of its New York City headquarters in 1980.

Although Revlon had already begun to look into possible alternatives to the rabbit eye-irritancy test, it made a grand gesture to calm the public waters: The firm announced a three-year, $750,000 grant to Rockefeller University to fund "an exploratory investigation" into the feasibility of developing a nonanimal alternative to the Draize test. Momentum picked up as other well-known firms, like Bristol-Myers and Avon Products, made large contributions to the alternative-test research kitty. A $1 million fund set up by the Cosmetic, Toiletry & Fragrance Association (CTFA) was used to create the Center for Alternatives to Animal Testing at Johns Hopkins University in Baltimore. Thus, university research to find alternative tests began in earnest.

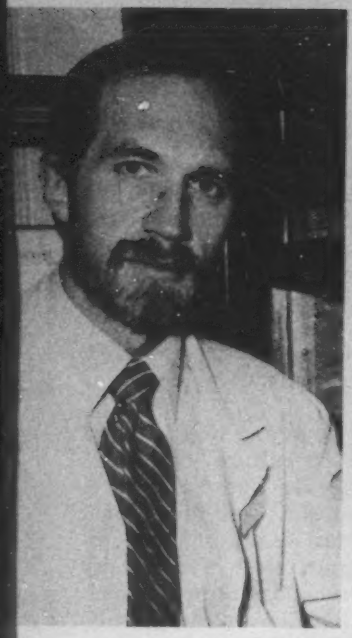
At Rockefeller's Laboratory Animal Research Center, Stark and colleagues Ellen Borenfreund, Charles Shopsis, and James Walberg are developing and testing a series of assays that could serve as a possible alternative to the Draize test.

A successful alternative, they explain should satisfy several requirements. First, the test must be easy to standardize, so that data from different labs can be reproduced readily. Second, it must be able to detect toxicity over a wide range of different chemical structures and target tissues. Third, the alternative should be able to evaluate the toxicity of complex mixtures. And fourth, it should be able to indicate whether recovery from the toxic insult is possible.

The assays under development at Rockefeller take several different approaches, but in general, they try to mimic several elements of the inflammatory response of the rabbit's eye in vitro. The process of inflammation is still not completely understood, but many of the structural and chemical changes that accompany eye irritation have been identified.

Biochemist Borenfreund, for example, is examining several different cultured cell lines for characteristic markers of irritation. These markers, which include synthesis of tissue-specific proteins and changes in cell shape and cell clustering, provide much more sensitive and subtle signals of toxicity than the biological endpoints, such as cell death, that toxicologists traditionally have used. In Borenfreund's assays, the endpoint is not cell death, but the highest tolerated dose—the concentration of toxicant that produces only minimal cellular changes.

In a similar vein, Shopsis has developed a sensitive assay based on a biochemical marker—the uptake of uridine into cultured mouse fibroblast cells. Uridine is a nucleoside that cells use to make RNA. Cells in culture will absorb uridine from the medium at a



(Clockwise from top left) Rockefeller's Walberg, Stark, Borenfreund, and Shopsis are developing a battery of assays that could be used as a possible replacement for the rabbit eye-irritancy test

measurable rate. This rate is a sensitive indicator of the health of the cell and the functioning of its membrane. When an irritant is added to the cell culture, Shopsis explains, the chemical interferes with transport of uridine through the membrane. Using radiolabeled uridine, Shopsis can determine the concentration of the irritant that causes uridine uptake to be inhibited by 50%. This value is used to rank the test substances according to their potency as irritants.

Shopsis tested more than 40 different substances using this assay and has found that his ranking correlates beautifully with Draize test scores for the same chemicals. What's more, Borenfreund's results using different cell markers also correlate with Shopsis', strengthening the notion that many different markers could be used to determine a chemical's irritancy potential in vitro.

The Shopsis assay also can measure the cells' recovery from the toxic insult. If a nonlethal concentration of irritant is used, its removal from the culture medium eventually will allow full membrane function to resume, as evidenced by a normal rate of uridine uptake.

Stark's contribution to the Rockefeller battery of alternative assays is, he admits, "farthest back on the burner," but it is no less intriguing. His assay involves a different aspect of the in vivo inflammatory response—the migration of macrophages to injured tissue. Macrophages are large white blood cells that engulf foreign material and move in response to the presence of certain chemical agents. The agents that attract macrophages are induced wherever inflammation occurs. Thus, regardless of the chemical identity of an irritant, if it produces inflammation, macrophages will be attracted to the injured site.

Stark wants to determine whether this migratory response, as measured in vitro, is correlated with irritancy observed in the live animal. To do this, he is studying how cultured macrophages react to fluids from mouse cell cultures that have been exposed to various irritants. Fortunately, the identities of the macrophage-attracting compounds present in these fluids needn't be known for the assay to work, Stark notes. His hope is that the migratory response, quantitated using special equipment, will correlate with the Draize scores of the irritants.

Unlike the other members of the Rockefeller team, veterinary pathologist Walberg is working with live rabbits. He has developed an apparently painless modification of the Draize test that provides objective data. Walberg exposes the eye of a rabbit to a dilute solution of a test compound. He then rinses the eye with distilled water after one hour and at predetermined time intervals thereafter. The cells that are washed out of the eye are counted and examined under the microscope.

In a series of tests using various alcohols of known irritancy, Walberg found that the number of such

exfoliated cells obtained at various time intervals after exposure to the alcohols paralleled, in general, the Draize test scores. The Draize scores were obtained by visually assessing inflammation in the treated eyes at the same times the eye rinses were done. In the case of one strong irritant, the cell count actually predicted acute, severe inflammation earlier than the Draize assessment.

Walberg's modification is not intended to replace the Draize test. Rather, he hopes it will "provide more objective data upon which validation procedures for alternative methods can be based." But animal activist Spira would like to see Walberg's test used as a substitute in corporate labs, at least until a validated in vitro alternative is available. Spira already has begun to put the pressure on companies to take a long, hard look at the Walberg test.

Walberg himself does not oppose Spira's tactic. Whether his cell exfoliation procedure is used as a Draize replacement or as a tool to help validate in vitro alternatives, its use, he says, is likely to reduce the need for repeated testing and decrease the number of animals used.

Researchers elsewhere are using different cell culture systems and a variety of methods to ascertain how well a chemical's irritancy potential can be predicted in vitro. For instance, at the University of Washington, Seattle, assistant professor of ophthalmology Kwan Y. Chan is focusing on rabbit corneal epithelial cells in culture. His assay involves measuring levels of plasminogen activator, an enzyme that is secreted in large amounts when cells in the cornea are injured or undergo drastic structural changes.

Chan's project, which is funded by the Johns Hopkins Center for Alternatives to Animal Testing, began only last May. Presently, he is trying to establish whether release of this enzyme can be used to signal cell damage caused by a chemical irritant such as bleach.

At about the time Chan was beginning his project, William H. J. Douglas and Stanley D. Spilman were finishing theirs at Tufts University School of Medicine in Boston. The Tufts researchers used cultured endothelial cells from calves' eyes. They first incubated the cells with sodium chromate labeled with radioactive chromium-51, which binds to the cells. Then the cells were exposed to a test substance and the amount of chromium-51 released by the cells was measured. Douglas and Spilman tested 56 substances in this way and ranked them by their potency to injure cells, as determined by the degree of radioisotope release. This in vitro ranking, they say, generally correlates with the substances' irritancy potentials as determined in vivo.

Douglas and Spilman were planning to go on and see how their assay worked on cultures of human corneal endothelial cells. However, both researchers left Tufts earlier this year, and as a result of personal difficulties on the part of Douglas, it's uncertain whether their study will be continued elsewhere. In any case, the New England Anti-Vivisection Society, which had funded the project, has disseminated their results to leading toxicologists around the U.S.

Although Douglas thinks the ideal in vitro replacement for the Draize test must rely on human corneal tissue, research suggests that corneal cells aren't essential to mimic eye inflammation or damage. Rockefeller's Borenfreund, for example, has studied the effects of about 40 chemicals, including alcohols, ketones, surfactants, and drugs, in five cell lines from different organs and species, including rabbit epithelial corneal cells. She found that the toxicity rankings of the compounds agreed very well in all the cell cultures, as well as with Draize scores.

But cell cultures are not the only in vitro methods that can be used to screen chemicals for irritancy. In fact, Joseph Leighton, an experimental pathologist at the Medical College of Pennsylvania, Philadelphia, takes a stronger view. He believes that tissue culture models are inadequate for simulating the inflammatory response in the body. Inflammation is expressed in a major way by changes that occur in blood vessels, he explains. In the Draize test, the inflammatory response involves the blood vessels in the rabbit's eye. But cell cultures lack blood vessels.

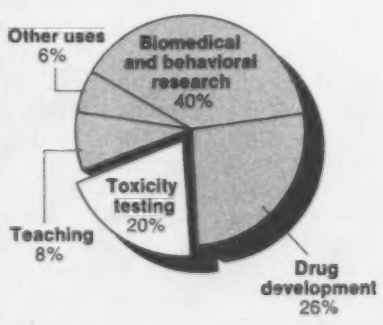
So Leighton chose to study irritancy in a tissue rich in blood vessels: the chorioallantoic membrane of chick embryos. This membrane forms under the shell during the incubation of the chick and serves as "a stand-in lung" for the embryo, he says.

With major support from the New York City-based American Fund for Alternatives to Animal Research, Leighton has been testing household products on the membrane. The membrane responds to chemical irritants like a living tissue—which it is—rather than like a cell culture. But because the membrane lacks nerves, the embryo feels no pain.

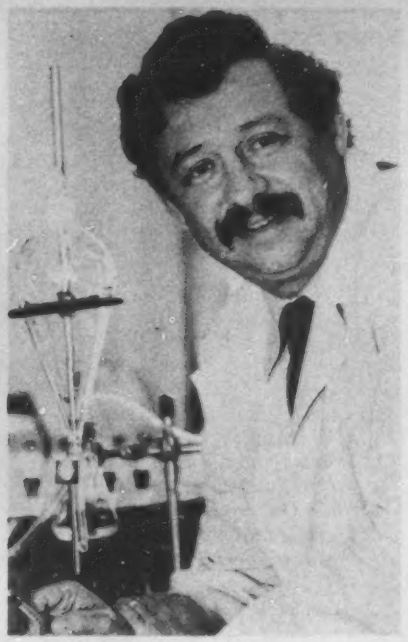
Rodents are most commonly used lab animals...

Animal	Number[a] (in thousands)
Mice	45,000
Rats	15,000
Birds	5,000
Frogs	3,000
Hamsters	1,000
Guinea pigs	1,000
Rabbits	750
Dogs	250
Ungulates	200
Cats	100
Primates (nonhuman)	25
TOTAL	71,000

...and 20% of all lab animals end up in toxicity testing



a Average number of laboratory animals used per year in research and testing in the U.S.
Source: Office of Technology Assessment



Chemical applied to chorioallantoic membrane causes severe reaction in circle (above) in work being done by Leighton (left). Goldberg's animal alternatives center at Johns Hopkins is funding similar research

So far, Leighton's bioassay can readily pick up severe irritants. But the test has difficulty in distinguishing between moderate and mild irritants in the Draize test. "We've a lot of work to do there," he says. His test could be useful in screening out severe irritants.

Recently, Colgate-Palmolive has shown interest in Leighton's work and has given him support. The firm plans to collaborate with him on a study that will check how reproducible his assay's results are, and how well they correlate with rabbit-eye data.

Although university research on Draize alternatives is highly visible, the industry that traditionally has depended on animal toxicity tests before marketing its products is making serious in-house efforts to find nonanimal test alternatives. These efforts are carried on largely behind the scenes because companies that make cosmetics, household products, and other consumer items usually consider their own self-developed test methods to be proprietary.

For example, Johnson & Johnson Baby Products has spent "a great deal of time and money" in the past six years to develop in vitro screening procedures to weed out harsh ingredients, according to irritancy researcher John J. McCormack. At a bioassay symposium held in 1981, McCormack presented details of an eye-irritancy test that he developed using mast cells from rat peritoneal tissue. That particular corporate disclosure, however, was exceptional. J&J rarely reveals such information, he explains, because it could erode the firm's "enormous advantage" in marketing mild products. The firm no longer uses the mast-cell test, he tells C&EN; it has been replaced by proprietary tests that presumably are superior.

Many other companies also screen out severely irritating products by in vitro methods, long before those products reach the animal testing stage. But in that penultimate stage, corporate testing labs have found that, for the time being, in vivo tests are unavoidable to ensure their products are safe for human use.

Even so, the trend is toward using as few animals as possible and minimizing their stress. At Procter & Gamble, for instance, scientists are using a refinement of the Draize test that calls for one tenth the amount of test substance normally used. Revlon also tests products in the rabbit eye in reduced quantities. Moreover, Revlon technicians sometimes rinse the rabbit's eye almost immediately after a test material has been applied. Some labs, like Avon Products', also administer local anesthetics to rabbits to reduce their discomfort. Most firms have eliminated repetitive tests. Avon has cut animal use by testing only final formulas, not interim ones. And Revlon has reduced the number of rabbits used more than 30%.

Besides these efforts, the cosmetic industry as a whole is funding outside studies that are delving into the basic mechanisms of inflammation and other toxicological responses. Much of this research is being sponsored by the Johns Hopkins Center for Alternatives to Animal Testing. The center, which materialized in 1981, already has assigned 16 grants to independent research groups: 14 in the U.S. and two in England. Of the U.S. projects, eight are clustered at Johns Hopkins.

Director Alan M. Goldberg, a neurotoxicologist, says that these research programs, which ultimately are aimed at developing alternatives to the Draize and other tests, are geared to exploring "areas of toxicity that are generally not being looked at by other groups."

The projects, Goldberg notes, are "highly focused" in three areas: responses of specific cell systems, structure and function of membranes, and secretory responses of cells and organs. The results coming out of these studies, he says, will provide the fundamental information necessary to develop a new generation of animal-free toxicity tests.

The Johns Hopkins animal alternatives center came into being, in large part, as a result of pressure from animal-welfare groups and individuals who deplored

C&EN staff photo

Animal-rights activist Spira (right) is crusading against the LD_{50} test. A statistical model of the LD_{50} developed by Enslein (far right) of Health Designs is reducing the need for the live-animal test

large-scale animal use in the Draize test. Once they were satisfied that positive steps were being taken to reduce the cosmetic industry's reliance on rabbits, animal advocates swiveled their guns on yet another toxicity test they saw as an outrage: the LD_{50}. The controversy surrounding this test quickly enveloped the scientific and regulatory communities.

The LD_{50} (for Lethal Dose-50%) is designed to determine the single dose of a test chemical that will kill 50% of the animals under study. It involves giving several groups of animals, usually rodents, different dosages of the chemical and counting how many die within a given time period. Statistical methods are used to convert this body count into an LD_{50} value.

This method has been used to evaluate the acute toxicity of a diverse range of substances, including cosmetics, detergents, pesticides, food additives, drugs, and other household and industrial products.

Toxicologists have found this test useful to gauge the relative acute toxicities of different chemicals and to set appropriate dosages for longer-term animal studies. And consumer-products companies have used it to assess how safe their products are. They explain that they need to know what a particular product will do if, for example, a child accidentally ingests it or spills it on the skin. They are obligated on moral grounds to do this, they insist.

But they are also bound by a legal obligation: Federal agencies such as the Food & Drug Administration, the Environmental Protection Agency, the Consumer Product Safety Commission, and the departments of Transportation, Agriculture, and Energy require that companies determine the acute toxic effects of their products. Some regulations, such as DOT's and EPA's, stipulate the LD_{50} as a requirement. But other regulations merely "recommend" the LD_{50} as a means of satisfying the requirement for acute toxicity data. In the past, many testing organizations have chosen, either because of convenience or misunderstanding, to comply with unspecific regulations by obtaining the required data using the universally accepted LD_{50} test.

But a growing number of scientists now believe that the classic LD_{50} test, developed in 1927, is "an anachronism," in the words of David P. Rall, director of the National Toxicology Program.

Activist Spira, who's led the assault on the LD_{50}, calls the test "a wasteful ritual exercise in misleading, meaningless precision." The test consumes a staggering number of animals each year, yet the numerical index of toxicity derived from it "is not terribly meaningful," echoes Goldberg of Johns Hopkins.

According to toxicologist Gerhard Zbinden of the Swiss Federal Institute of Technology, the LD_{50} value for any compound is not a biologic constant and its determination is subject to enormous variability from laboratory to laboratory. In fact, he and many other scientists believe that a highly precise LD_{50} determination is rarely necessary. The classical test typically uses 40 to 200 animals at one time. Yet the same information, he says, could be gleaned using fewer than a dozen animals.

The regulatory agencies have attracted much of the anti-LD_{50} fire. "A simple change in regulatory requirements could, almost overnight, reduce animal use in the LD_{50} test by 4.5 million animals—a 90% reduction," Spira asserts. "The real villain is the inertia inherent in any large bureaucracy. To overcome this inertia ... requires enormous public pressure."

In his drive to build "a fire of public opinion under the regulatory agencies," Spira is getting support from several industry organizations. The Pharmaceutical Manufacturers Association, for example, has called for regulators to phase out the LD_{50}. It also has urged its member companies to use alternatives to the test. CTFA's board of directors also has joined the hue and cry and encouraged cosmetic companies to limit their use of the LD_{50} whenever it's not essential.

Some federal bureaus and toxicology programs no longer use, or require the use of, the classic LD_{50} test. But animal activists are pushing strongly for a total abolition of the test.

In response to this rising tide of opposition, FDA has scheduled a symposium on Nov. 9 that will exam-

ine regulatory requirements for the LD_{50}. The basic reason for the meeting, explains coordinator Phillip A. Johns, is to clarify for the public what the federal agencies' toxicity testing requirements really are. Johns, a toxicologist with FDA's Office of Science Coordination, says the public's confusion is mirrored by the animal advocates' newspaper ads implying that FDA is requiring "poor little dogs to be killed" in LD_{50} studies. In fact, he states, "we have not required or even suggested that anybody use dogs in an LD_{50} study for at least 35 years. It's almost a defamation of our agency."

According to industry sources, use of the classic LD_{50} test is reduced significantly already. Several modifications of the test that use far fewer animals have become ensconced in corporate testing labs. At Procter & Gamble, for instance, biostatistician Robert D. Bruce has developed an "up-and-down" method to pinpoint a chemical's acute toxicity. In this test, animals are dosed one at a time. If an animal survives for one or two days, the dose for the next animal is upped; if it dies, the dose is lowered. A computer program converts the data into an estimated LD_{50}. P&G says this procedure usually requires only six to 10 animals and gives results that are in "excellent agreement" with the standard test.

In certain cases, computer-generated mathematical models can estimate the LD_{50} of a new compound from its chemical structure and properties. According to Kurt Enslein, who heads a company called Health Designs in Rochester, N.Y., such predictive models can provide reasonably good LD_{50} estimates for about 80% of the compounds he has looked at.

To fashion Enslein's model for the rat oral LD_{50}, for example, a computer was used to pore over data from more than 6000 compounds. Using statistical methods, it culled the particular structural features and physical and chemical properties of the molecules that seemed to be most important in influencing their LD_{50}s, which were obtained from published studies. By plugging this same type of data for a new molecule into the equation, one can arrive at an estimate of the compound's oral LD_{50} in rats.

Enslein now has received support from an animal welfare group to refine this mathematical model so that it could be accepted by DOT as a substitute for the LD_{50} test. If his LD_{50} model is put into use, Enslein predicts, more than half of the compounds submitted for DOT approval will not need to be tested in animals.

Although recent public outcry has focused on the Draize and LD_{50} tests, scientists have been working successfully for years to reduce animal use in the testing of chemicals' long-term toxicity. Such chronic effects are insidious, often not surfacing until years or decades after the original exposure. For example, chemicals can cause chromosomal mutations that may appear as cancer or genetic diseases. Chemicals also can give rise to birth defects, cardiovascular disorders, and nervous system and behavioral problems.

Probing a new substance for mutagenic, carcinogenic, and teratogenic effects, until fairly recently could involve using hundreds, even thousands, of rodents. A lifetime study (18 to 24 months) of a chemical in one species of animal could cost $300,000 or more, says biologist Roland M. Nardone of Catholic University of America, Washington, D.C.

But in vitro methods using bacteria, cell and tissue cultures, fruit flies, and similar biological systems increasingly are allowing toxicologists to screen out "bad actors" at an early stage without resorting to live animals. At Procter & Gamble, for instance, a first-level mutagenicity screening consists of a battery of tests including the Ames bacterial assay, a mouse lymphoma assay, and other in vitro tests using hamster ovary cells and rat liver cells in culture.

Even neurotoxic effects can be tracked down without using live animals. At Catholic University, Nardone is developing an in vitro battery of tests to detect potential neurotoxicity of chemicals.

But in the realms of drug development and biomedical and behavioral research—where about two thirds of all lab animals are used—the outlook for replacing animals is dim. Donald G. Stein, director of the brain research laboratory at Clark University, Worcester, Mass., says it is "naive reductionism" to think that the behavior of a complex, living organism can be generalized from a layer of cells in a petri dish. Stein, who is using mice and rats to learn how to promote recovery from brain injury in humans, says that live animals are essential in his research, and will continue to be in the foreseeable future.

Many animal welfare groups, however, adamantly reject such views. For example, Aaron Medlock, executive director of the New England Anti-Vivisection Society, says that alternatives to animal experimentation could be found if scientists only tried hard enough.

The controversy enveloping the use of live animals in testing and research perennially surfaces on Capitol Hill. In the 98th Congress, two major bills address the issue. One proposal, sponsored by Sen. Robert Dole (R.-Kan.), would amend the Animal Welfare Act. His bill, designated S. 657, would provide researchers better access to information on improved methods of animal experimentation, including ways to reduce or replace animal use.

Dole's proposal, which is still in committee, also contains controversial provisions that some fear might lead to federal intrusion into the lab. Sen. Orrin G. Hatch (R.-Utah) terms the Dole bill "disastrous" and has introduced his own, S. 773. The Hatch bill calls for a comprehensive assessment of the current status of the use of live animals in biomedical and behavioral research. It is awaiting action on the Senate floor.

According to Hatch, it's unclear which alternatives to the use of animals should be promoted. This week, at his request, the Office of Technology Assessment is beginning an 18-month study to examine this question and other issues regarding animal experimentation and testing. The OTA study is expected to provide Congress with information necessary to consider further legislation in this area. And further legislation undoubtedly will be required, for no one expects animal testing, and the controversy surrounding it, to disappear any time soon. □